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HYBRIDIZATION AND GERM CELLS OF OENOTHERA MUTANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY

XCIII

REGINALD RUGGLES GATES

Since my previous paper[1] went to press, in which were described the conditions of abortive pollen development in *O. lata*, as well as some of the stages in pollen development in the *O. Lamarckiana* hybrid obtained by crossing *O. lata* with *O. Lamarckiana* pollen, further investigations have placed in a new light the situation in regard to the number of chromosomes in the Oenothera mutants and hybrids studied. The further facts obtained have disclosed quite a different condition from the one anticipated, and one which is at present wholly inexplicable, and apparently quite unique so far as chromosomes are concerned, though it is to be expected that fuller knowledge of the facts will bring an explanation of the situation. Since the facts, so far as known at present, appear to be most directly concerned with Oenothera hybrids, a brief account of the forms of hybridization among the Oenothera mutants, as developed by the very extensive hybridizing experiments of DEVRIES,[2] will first be necessary.

FORMS OF OENOTHERA HYBRIDS

In general, DEVRIES' experiments appear to disclose at least three main forms or types of hybridization in the Oenothera mutants: (1) hybrids between a mutant and the parent *O. Lamarckiana;*

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[1] GATES, R. R., Pollen development in hybrids of *Oenothera lata* × *O. Lamarckiana*, and its relation to mutation. BOT. GAZETTE 43:81–115. *pls. 2–4.* 1907.

[2] DEVRIES, HUGO, Die Mutationstheorie, Vol. II. 1903.

(2) hybrids between two mutants; (3) ordinary Mendelian hybrids, which appear in some of the mutant crosses. These can only be considered as general tendencies, however, for the results vary widely in many of the crosses, both in the number and the percentage of the types appearing. DeVries has also been able to vary the results experimentally in a number of cases. Crosses in which a mutant is involved he calls mutation hybrids (*Mutationskreuzungen*), and hybrids of exactly this character do not occur, so far as I know, in any other genus of plants or animals.

The conspicuous types of hybridization in Oenothera are as follows: When *O. Lamarckiana* is crossed with a mutant, in the case of several of the forms DeVries experimented upon, a hybrid is produced which splits, but differs from a typical Mendelian hybrid in three respects. (1) A heterozygote, showing only the characters of the dominant parent, and containing the recessive characters latent, is not formed in the first hybrid generation, but instead the two parent forms are produced. (2) The proportions of the offspring are not always about 3:1 as in a Mendelian hybrid, but the percentage of plants having the recessive characters (the mutants are recessive to the parent *O. Lamarckiana*) often fluctuates between 0 and 50 per cent., or even with wider range. This distinction is believed to be less important, as fluctuations in the percentage of the forms are known to occur in some Mendelian hybrids. (3) A still more striking difference is that, while in a Mendelian hybrid two-thirds of the dominant plants continue to split in the proportions of 3:1 in successive generations, both the forms produced in a mutation hybrid, or cross of *O. Lamarckiana* with a mutant, breed true and show no splitting. There is thus no external evidence that crossing has ever occurred. This type of hybrid appears when *O. lata*, *O. nanella*, or *O. rubrinervis* is crossed with *O. Lamarckiana*. The following diagram will make clear the distinctions between this and an ordinary Mendelian hybrid. *A* represents the characters of *O. Lamarckiana* in *fig. 1*, and of the dominant parent in *fig. 2*. *B* represents the characters of a mutant in *fig. 1*, and of the recessive parent in *fig. 2*.

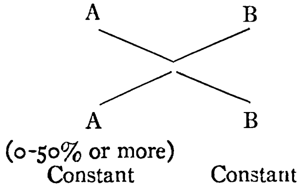

FIG 1.—Mutation hybrid

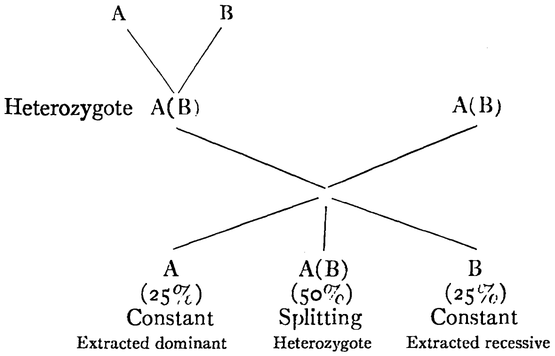

FIG. 2.—Mendelian hybrid

Two significant exceptions to this form of hybrid among the mutants are found in *O. brevistylis* and *O. laevifolia*, which never appeared as mutants in DEVRIES' cultures, although he believes them to have formerly mutated from *O. Lamarckiana*. They form with *O. Lamarckiana* ordinary Mendelian hybrids.

The fact that the percentage of a mutant appearing in a cross can be experimentally varied in different ways is also of importance in this connection. Thus the percentage of the parent forms appearing in a cross varies with the position on the stem of the particular flowers crossed. The plants bloom from below upward on the stem, and DEVRIES observed that after the first few weeks of blooming there is a steady and marked decrease in the size of the flowers blooming, until at the close of the season they are but little larger than those of *O. biennis*. I have observed the same thing in my own cultures from seeds of DEVRIES at Woods Hole, Mass. At the end of September many of the plants were still blooming vigorously at the top, and the flowers were little if any larger than those of *O. biennis*. When pollen from a mutant is used to cross with *O. Lamarckiana*, if it is obtained from the earlier flowers of the season, that is from the larger and more vigorous flowers which are lower on the stem, the heredity-coefficient,[3] or percentage of the mutant appearing in a cross, will be larger than if later and smaller flowers are used. Similarly the heredity-coefficient (*Erbzahl*) of flowers on the side branches of *O. lata* when crossed with *O. Lamarckiana* is said to be less than that of flowers on the main axis (DeVries *l. c.* **2**:416). The heredity-coefficient of a mutant, when the large earlier flowers are used, appears to be a fairly constant quantity and to be usually or at least frequently near the Mendelian ratio, though it may often fluctuate in individual crosses of flowers from 0 to about 50 per cent. Thus the heredity-coefficient of *O. lata* is often 21–24 per cent., and that of *O. nanella* is practically the same. This percentage is said to remain about the same from generation to generation and to be independent of the pollen parent; but in crosses of single flowers the percentage may vary widely. Thus 39 crosses of *O. Lamarckiana* flowers were made with *O. nanella* pollen, and the seeds from the different flowers sown separately. The proportions of *O. nanella* obtained, that is the heredity-coefficient, varied from 1 to 48 per cent., the average for the total number of 3768 seedlings being about 17 per cent. (DeVries *l. c.* **2**:408).

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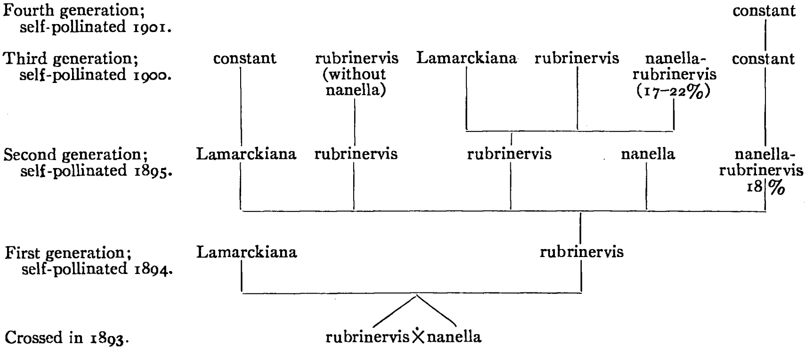
[3] I would suggest this term as an English equivalent for the German *Erbzahl* and shall use it in this sense in the rest of the paper.

The proportions of the parents appearing in a cross also fluctuate from other causes. In crossing *O. Lamarckiana* with *O. nanella* pollen, in one experiment when 90–100 seedlings were produced from one ovary the proportion of *O. nanella* was 44 per cent., but when the number of seedlings produced from an ovary was only 20–40, the proportion of *O. nanella* was reduced to 10 per cent. The quantity of pollen used in a cross also influences the offspring, and the proportions may be varied by experimental methods. Thus in one experiment (DeVries *l. c.* **2**:417), all of the 4–8 lobes of the stigma of *O. Lamarckiana* flowers were removed except one lobe or a small portion of one. They were then rather sparingly pollinated with *O. nanella* pollen (one flower being used to pollinate several), and of 1593 seedlings produced from 8 *O. Lamarckiana* plants whose flowers were thus treated, all were *O. Lamarckiana.* In another experiment all of the stigma was removed but one lobe, and of 1687 seedlings obtained only about 2 per cent. were *O. nanella.* Other experimental means of varying the results of a cross need not be mentioned here. But that such results can be obtained is of the greatest interest and suggests a line of inquiry as to the nature of the factors determining what forms will appear in a cross. This may also have a bearing on the determination of the chromosome number, though just what the connection may be cannot be guessed at present.

When two mutants are crossed the results show a much greater variation in numbers and forms. In some cases four types are produced in the F_1, though one or more of these types may be absent in any cross. *O. lata*×*O. nanella* may be taken as an example. In one experiment[4] the F_1 showed: *O. lata* 30 per cent., *O. Lamarckiana* 18 per cent., *O. nanella* 47 per cent., *O. nanella-lata* 5(?) per cent. The constant intermediate hybrid type *O. nanella-lata* is usually absent from this cross, and the percentage of the other types may vary widely. The *O. Lamarckiana* and *O. nanella* hybrids from this cross breed true, as does also *O. nanella-lata* when present. Since *O. lata* does not mature its pollen, its constancy cannot be tested by self-pollination.

One other instance of a cross between two mutants (*O. rubrinervis*×*O. nanella*) will be given, to show the complexity and variety of the results.


Fourth generation; self-pollinated 1901.
constant
Third generation; self-pollinated 1900.
constant
rubrinervis (without nanella)
Lamarckiana
rubrinervis
nanella-rubrinervis (17–22%)
constant
Second generation; self-pollinated 1895.
Lamarckiana
rubrinervis
rubrinervis
nanella
nanella-rubrinervis 18%
First generation; self-pollinated 1894.
Lamarckiana
rubrinervis
Crossed in 1893.
rubrinervis×nanella

In this case (DeVries *l. c.* **2**:451) *O. Lamarckiana* and *O. rubrinervis* were produced in the first hybrid generation, the latter on self-pollination producing (1) *O. Lamarckiana*, which was constant; (2) *O. rubrinervis*, part of which were pure and part split into *O. Lamarckiana*, *O. rubrinervis*, and *O. nanella-rubrinervis* as in the previous generation; (3) *O. nanella*, whose constancy was not tested; (4) *O. nanella-rubrinervis*, which bred constant through three generations. The form *O. nanella-rubrinervis* is an intermediate type between *O. rubrinervis* and *O. nanella;* it has never appeared as a mutation or in the first hybrid generation, but apparently arises only from self-fertilized hybrids of the second or later hybrid generations.

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[4] DeVries, Hugo, Ueber erbungleiche Kreuzungen. Ber. Deutsch. Bot. Gesells. **18**:435–443. 1900.

In these crosses it should be said that, while constancy of the forms appearing in the F_1 is the rule, exceptionally in one of the types which ordinarily breeds true an individual may appear whose progeny repeat the two types of the grandparents, thus showing a tendency to continue splitting (DEVRIES *l. c.* **2**:423).

SHULL'S[5] recent work on hybridization in the elementary species of Bursa (Capsella) shows that these produce ordinary Mendelian hybrids in no way comparable to Oenothera hybrids. In fact I think it is safe to say that no genus of plants or animals is known in which the conditions of hybridization are strictly comparable to those found in Oenothera. This peculiar type of hybridization is not confined to *O. Lamarckiana* and its mutants, but ordinarily appears when a mutant is crossed with another member of the genus, as *O. muricata*, *O. biennis*, *O. hirtella*, *O. suaveolens*, *O. hirsutissima*. In such cases the first hybrid generation usually contains both the parent forms in widely varying proportions, and these in the F_2 are found to breed true. Thus the same type of hybrid is produced as is usual when a mutant is crossed with *O. Lamarckiana*.

The phenomena of hybridization in Hieracium are more nearly like those of Oenothera than any other genus, so far as I know. MENDEL'S[6] original work, continued recently by OSTENFELD,[7] has given us a view of the hybridization conditions in this genus. MENDEL showed in a number of Hieracium crosses that the F_1 is heterogeneous, including some intermediate types and some very near to or indistinguishable from the parents. In the case of *H. praealtum* × *H. flagellare*, the F_2 obtained by self-pollinating the hybrids of the F_1 was shown to be the same as the F_1, all the forms of the latter breeding true. OSTENFELD[8] first showed the presence of apogamy in the genus by castration experiments. MURBECK[9] has since found that the egg in these cases develops the embryo parthenogenetically. The absence of fertilization in some cases is thus a factor at least in the peculiar form of hybridization. OSTENFELD[7] has recently extended these studies to a considerable number of species. Among other species he has shown by castration experiments, confirmed by the cytological investigations of ROSENBERG,[10] that *H. excellens* is partly parthenogenetic and partly develops normally fertilized eggs; its pollen is also sterile. ROSENBERG found in this form the interesting condition which he calls apospory, where a cell of the nucellus enlarges and obliterates the normally formed tetrad of megaspores. This cell completes the embryo sac morphology and the egg develops without fertilization. More rarely apogamous embryo sacs are formed, as in Taraxacum. Usually, however, normal tetrad formation, with reduction of chromosomes, takes place, followed by fertilization. Part of the plants produced in a cross are then pure *H. excellens* produced from apogamous or aposporous embryo sacs and part are real hybrids.

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[5] SHULL, G. H., Elementary species and hybrids in Bursa. Science **25**:590–591. 1907.

[6] MENDEL, G., Ueber einige aus künstlicher Befruchtung gewachsene Hieracium-Bastarde. Verh. Naturf.-Vereins. Brünn. **8**:26–31 (Abhandlungen). 1869.

[7] OSTENFELD, C. H., Castration and hybridization in the genus Hieracium. Rep. 3d Internat. Conf. on Genetics. Jour. Roy. Hort. Soc. London **24**:285–288. 1900.

———, Experimental and cytological studies in the Hieracia. I. Castration and hybridization experiments with some species of Hieracia. Bot. Tidssk. **27**:225–248. *pl. 1.* 1906.

The similarity of this form of hybrid to the *O. lata*×*O. Lamarckiana* cross suggests the presence of apogamy in *O. lata* also, though it is highly improbable that this can explain all the phenomena of hybridization concerned. In the latter cross only the two parent types are produced and the *O. Lamarckiana* breeds true, while in the Hieracium cross several types are produced intermediate between the parents, in addition to the pure *H. excellens*, but experiments of OSTENFELD have not yet been carried far enough to determine the character of later generations, and the number of plants he has obtained from seeds has been in some cases too small for entirely satisfactory results.

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[8] OSTENFELD, C. H., Zur Kenntnis der Apogamie in der Gattung Hieracium. Ber. Deutsch. Bot. Gesells. **22**:376–381. 1904.

———, Weitere Beiträge zur Kenntnis der Fruchtentwickelung bei der Gattung Hieracium. *Idem* 537–541.

[9] MURBECK, S., Parthenogenese bei den Gattungen Taraxacum und Hieracium. Bot. Notiser **1904**:285–296.

[10] ROSENBERG, O., Ueber die Embryobildung in der Gattung Hieracium. Ber. Deustch. Bot. Gesells. **24**:157–161. *pl. 11.* 1906.

[10a] ———, Experimental and cytological studies in the Hieracia. II. Cytological studies on the apogamy in Hieracium. Bot. Tidssk. **28**:143–170. *pls. 1–2.* 1907.

The Oenothera and Hieracium hybrids mentioned may be said to resemble each other in that in both the F_1 is heterogeneous, though the types of which the F_1 is composed differ in the two crosses, agreeing only in that one of the types in each is the pure mother parent. The abortive character of the pollen in *O. lata* may also be said to favor rather than otherwise the probability of apogamy in this species, as it is well known that apogamous species frequently do not mature their pollen. This suggestion of apogamy in *O. lata* will be examined in another light in connection with the discussion of the number of chromosomes.

MILLARDET'S[11] "false hybrids" in Fragaria and Rubus, in which only one of the parent types (usually the mother) is produced in the hybrid, and this breeds true, may possibly be regarded as extreme cases of the same type of hybrid as some of the Oenothera crosses.

HURST,[12] among a large number of crosses of orchids which showed many types of hybridization, found eleven crosses between different genera in which only one of the parent types was reproduced, this being in every case the mother (p. 104). Large numbers of the hybrids were grown in some cases. He concluded that in at least eight of these "false hybrids" self-pollination was impossible, and that the embryos probably developed apogamously, perhaps on account of the stimulus from the presence of foreign pollen tubes in the ovary, but without fertilization. Oenothera itself may produce hybrids of this type. Thus DEVRIES found that *O. Lamarckiana* ♀ ×*O. biennis* ♂ gives only *O. biennis* in the F_1 and F_2 (*l. c.* **2**:31). This cross made in the New York Botanical Gardens, however, gave in addition to several *O. Lamarckiana* plants, which MACDOUGAL[13] thinks probably came from foreign *Lamarckiana* pollen, four types, none of which were the same as either parent. Thus entirely different results may be obtained under different conditions.

[11] MILLARDET, M. A., Note sur l'hybridation sans croissement ou fausse hybridation. Mem. Soc. Sci. Phys. et Nat. Bordeaux IV. **4**:347–372. 1894.

[12] HURST, C. C., Notes on some experiments in hybridization and cross-breeding. Jour. Roy. Hort. Soc. London **24**:90–126. *figs. 9–41.* 1900.

[13] MACDOUGAL, D. T., Mutants and hybrids of the Oenotheras. Publ. 24, Carnegie Institution, Washington, pp. 56. *pls. 22.* 1905.

The possibilities of apogamy in other mutants are apparently not so good, and such a condition seems rather unlikely, especially in *O. nanella*, where some of the hybridization results practically exclude the possibility of apogamy in these cases. Thus, *O. nanella* ♀ × *O. biennis* ♂ gave (DEVRIES *l. c.* **2**:476) in two crosses 554 plants, none of which were *O. nanella;* while the reciprocal cross gave 96–100 per cent. *O. nanella* among 356 plants. Hence in both cases the pollen parent was practically the only form appearing in the F_1, so that apogamous embryos, at least of any kind known at present, could not have been formed.

THE NUMBERS OF CHROMOSOMES

In my earlier paper[1] it was shown that the sporophyte number of chromosomes in *O. lata* is fourteen, while in the *O. Lamarckiana* plants appearing in the first hybrid generation from a cross with *O. lata*[14] the number is at least twenty. It was assumed therefore that the pure *O. Lamarckiana* parent had at least twenty chromosomes as the sporophyte number. Examination has shown, however, that in the pure *O. Lamarckiana* the sporophyte number is fourteen. This creates a remarkable and unexpected situation, which may be illustrated thus:

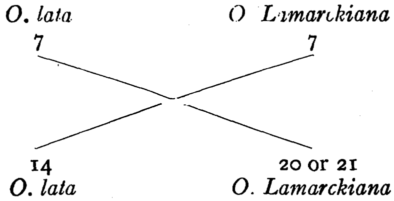

FIG. 3

Two plants, whose gametophyte number of chromosomes is seven, when crossed give rise in the first hybrid generation to the two parent forms, one of which has fourteen chromosomes as the sporophyte number, while the other has twenty or twenty-one.

In *O. lata* the count of chromosomes was made in the pollen mother cells and found to be fourteen. It has since been made in various somatic tissues of the flower, and is found to be constantly fourteen so far as observed. There has been no indication whatever that the number is ever higher. In the *Lamarckiana* hybrid it has been found from a number of counts in the floral tissues that the number of chromosomes in the somatic tissues is twenty or twenty-one, and I have found no reason to think it varies. In the pollen mother cells the number of chromosomes in the metaphase and anaphase of the heterotypic mitosis is usually twenty or twenty-one. Occasionally the number in early anaphase appears to be less, but this may be due to a tardy separation of some of the paired chromosomes in the equatorial plate; further study is required to determine this point. A large number of telophases of this mitosis were counted which showed ten chromosomes each. In one case there appeared to be eleven chromosomes in a telophase, though the greater majority at least show ten in each daughter nucleus. Occasionally a chromosome lags behind in the equator of the spindle until late anaphase, and this may make the number twenty-one. Such a case is figured in my former paper (*pl. 4, fig. 39*). This is not always the case, however, as shown by *fig. 38*, in which there are ten chromosomes in each daughter nucleus, but none left behind in the equator of the spindle. It is possible that a single unpaired chromosome might be present in metaphase and be lost in the cytoplasm as the paired chromosomes separated and went to the poles. It is certain that in many pollen mother cells ten chromosomes go into each daughter nucleus in the heterotypic mitosis. Whether the number in the somatic tissues is twenty or twenty-one has not been determined with certainty, as it is a matter of very considerable difficulty. The further discussion will reveal the reason for suggesting that the sporophyte number of chromosomes in the *Lamarckiana* hybrid is twenty-one rather than twenty.

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[14] For the sake of brevity I shall refer to the *O. Lamarckiana* plants appearing from flowers of *O. lata* pollinated by *O. Lamarckiana* as "the *Lamarckiana* hybrid."

O. Lamarckiana, the parent, has fourteen chromosomes as the sporophyte number, so far as the plants have been examined. This count was made constantly in the floral tissues. The homotypic mitosis in the pollen mother cell shows seven chromosomes constantly so far as observed, a large number of counts being made in various stages of this mitosis. It is doubtless true that chromosome counts as ordinarily made come from a very few individuals. In the case of the *Lamarckiana* hybrid I have examined material from two individuals and from different flowers in each; these plants were grown in different seasons. In every count the number of chromosomes in the somatic tissues has been twenty or twenty-one, though I have been as yet unable to determine with certainty between these two numbers. Any cytologist is acquainted with the fact that one region of a chromosome group is likely to be obscure enough, owing to close aggregation of the chromosomes, to prevent absolute certainty in making a count of the exact number, unless the number of chromosomes happens to be a small one. The evidence already mentioned shows beyond a doubt that, in the large majority of cases at least, ten chromosomes enter each daughter nucleus in the heterotypic mitosis, though it may be that there are occasionally eleven chromosomes in one of the daughter nuclei.

Several plants of *O. lata* and the pure *O. Lamarckiana* have been examined, all having fourteen chromosomes, which makes it probable that fourteen is the number found in all the pure individuals of these species, and twenty or twenty-one the number found in all the individuals of the *Lamarckiana* hybrid. But it will be necessary to determine the count in several more plants of each of these races before this point can be settled with certainty.

It will be of interest to know whether the number of chromosomes in the *Lamarckiana* hybrid remains constant in later generations, and also what happens when the *Lamarckiana* hybrid having twenty or twenty-one chromosomes is crossed with *O. lata* or with pure *O. Lamarckiana* having fourteen chromosomes. I am now growing plants from crosses made in last season's cultures, which it is hoped will determine these interesting points.

THE HETEROCHROMOSOMES

In my former paper[1] (p. 91), large rings of chromatin were described as sometimes occurring in the pollen mother cells of *O. lata*, cut off from the spirem after synapsis. In the heterotypic mitosis in *O. lata* and the *Lamarckiana* hybrid small bodies are found, ring-shaped (or hollow) but of the same size as the chromosomes, from which they are otherwise indistinguishable. These may be on the spindle or in the cytoplasm, as described in that paper. It was believed that the smaller ring-shaped chromatic bodies were condensed from the larger rings found in the spirem stage. It is possible, however, that the latter are merely irregularities occurring during pollen degeneration in *O. lata*, for it is well known that a variety of irregularities, in addition to the ones described, may occur in synapsis and the reduction mitosis in sterile hybrids. This leaves unexplained the origin of these bodies called heterochromosomes. On the other hand, the evidence from staining properties and from other considerations, as given in the paper referred to, is against their origin from nucleoli. The same bodies have since been found in the reduction mitoses in *O. Lamarckiana*, where they persist, in some cases at least, through both mitoses to the formation of pollen tetrads. For the present, therefore, the origin and possible significance of these bodies may be considered to be a matter of doubt, in regard to which no conclusions can be drawn until further evidence is obtained.

POSSIBLE EXPLANATION OF THE CHROMOSOME NUMBERS

A number of possible explanations of the origin of the chromosome number in the *Lamarckiana* hybrid will at once occur to botanists. It must be said, however, that all of them appear unlikely, and no explanation can be given on the basis of our present knowledge in regard to chromosome numbers. It is to be expected that further investigation will disclose the sources of the extra chromosomes in the *Lamarckiana* hybrid, but for the present some possibilities may be suggested.

It would seem more probable, because more in accord with present views of chromosomes, that the sporophyte number of chromosomes in the *Lamarckiana* hybrid arises at the time of fertilization by the union of nuclei the sum of whose chromosomes is twenty-one, than that it arose through the subsequent divisions of certain chromosomes. The experimental results of NĚMEC[15] and others, in which somatic nuclei are made to fuse and subsequently divide, showing mitotic figures with chromosomes whose number is the sum of the number of chromosomes in the nuclei that entered the original fusion, indicate the persistency of individual chromosomes in such cases. Such results increase the probability that the number of chromosomes found in the somatic tissues of an individual arose with the genesis of the individual and has persisted in all subsequent cell generations. Of course there is the possibility that a higher number of chromosomes might originate through the longitudinal division or the fragmentation of certain chromosomes; but no constant size-differences have been found among the chromosomes of any of the forms or between the chromosomes of the different races examined.

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[15] NĚMEC, B., Ueber die Bedeutung der Chromosomenzahl. Bull. Internat. Akad. Sci. Bohême **10**: 1–4. 1906.

There are a number of ways in which it would be possible for fusion nuclei having twenty-one chromosomes to be formed during the period of fertilization, since the gametophyte number of chromosomes is seven.

(1) It is possible that *O. Lamarckiana* might produce two kinds of pollen, having respectively seven and fourteen chromosomes. My examination of the conditions of pollen development in *O. Lamarckiana* has thus far revealed no evidence whatever in favor of such an hypothesis. Apparently all the pollen mother cells undergo the reduction divisions, in which the number of chromosomes is reduced from fourteen to seven in the usual way.

(2) It is possible that *O. lata* might produce two kinds of eggs, having respectively seven and fourteen chromosomes. If both these kinds of eggs were fertilized with *O. Lamarckiana* pollen and produced embryos, we should have plants resulting with fourteen and twenty-one chromosomes. The difficulty here, however, is that on such an hypothesis the union of seven *lata* chromosomes with seven *Lamarckiana* chromosomes would produce a *lata* plant; while the union of fourteen *lata* chromosomes with only seven *Lamarckiana* chromosomes would produce a *Lamarckiana* plant, a situation which is highly improbable, to say the least.

(3) Another possibility is that all the eggs of *O. lata* have the unreduced number of chromosomes, and that part of them develop without fertilization (parthenogenetically), producing *O. lata* plants with fourteen chromosomes; while others are fertilized with *O. Lamarckiana* pollen, and produce *Lamarckiana* plants having twenty-one chromosomes (*fig.* 3). This assumption is perhaps as reasonable as any, but no case is known of an unreduced egg being fertilized.

(4) Another possible source of twenty-one chromosomes is by the union of both male cells with the egg in fertilization. This, though very unlikely, is at least a theoretical possibility.

(5) It is conceivable that the triple fusion nucleus in double fertilization, instead of the egg, might form the embryo. The only known case of the endosperm developing anything resembling an embryo is Balanophora. TREUB[16] studied *B. elongata* and the same conditions were afterward found in *B. globosa* by LOTSY.[17] Fertilization does not take place in these forms, and the egg and synergids soon degenerate, while the upper endosperm nucleus enlarges and divides, forming a mass of endosperm tissue. In the midst of the endosperm a group of smaller cells is cut off, forming a "pseudembryo," which was followed to the five or ten cell-stage. Tetrad formation apparently does not take place, so that there is probably no reduction of chromosomes, the "pseudembryo" having the $2x$ number.

All of these possibilities, therefore, are without parallel in our present knowledge, but it seems most probable that the source of the twenty-one chromosomes is to be sought in some such situation during fertilization, or the genesis of the individual having this number of chromosomes. It is hoped that further investigation of megaspore and embryo sac development and fertilization in *O. lata* will lead to the explanation of this remarkable situation, though of course the real explanation may be quite different from any of the possibilities here suggested.

On any of these assumptions the number of chromosomes is twenty-one. If it is twenty, however, another explanation must be sought. The correctness of the latter number is supported by the fact that I have counted many telophases of the heterotypic mitosis, in which the number of chromosomes was ten in each daughter nucleus. Also if twenty-one chromosomes originated from the fusion of three nuclei, two of which represented one parent and one the other parent, it might be expected that in synapsis and the reduction mitosis a segregation of the chromosomes would indicate this origin. One might perhaps expect a pairing of seven chromosomes (maternal) with seven others (paternal), leaving the other seven unpaired to pass to either pole or remain out in the cytoplasm. ROSENBERG[18] found such a situation in the Drosera hybrid, where the ten chromosomes from one parent paired with ten of the twenty from the other parent, while the remaining ten chromosomes of the latter remained unpaired and were irregularly distributed or lost in the cytoplasm. But nothing of this kind has thus far been observed in the reduction divisions of the *Lamarckiana* hybrid, and instead ten chromosomes are apparently regularly distributed to each daughter nucleus in the heterotypic mitosis. It is pretty evident that the exact number of chromosomes cannot be satisfactorily settled until their origin is ascertained.

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[16] TREUB, M., L'organe femelle et l'apogamie du *Balanophora elongata* Bl. Ann. Jard. Bot. Buitenzorg **15**:1–22. *pls. 1–8.* 1898.

[17] LOTSY, J. P., *Balanophora globosa* Jungh. Eine wenigstens örtlich-verwittwete Pflanze. Ann. Jard. Bot. Buitenzorg II. **1**:174–186. *pls. 26–29.* 1899.

Not the least remarkable part of this situation is found in the fact that here we have two plants, the pure *O. Lamarckiana* and the *Lamarckiana* hybrid, which cannot be distinguished by their external characters, and yet differ widely in the number of their chromosomes. This difference in number assumes a different aspect, however, if we find, as seems most probable, that we have here merely an extra set of chromosomes from one parent. Such a situation need not necessarily interfere with the present views of persistency of chromosome numbers or even of the ordinary constancy in number for a species. A knowledge of the fate of these chromosomes in subsequent generations and crosses will probably aid largely in determining the general bearing of these facts on present views of the individuality and constancy in number of chromosomes.

It is suggestive that in the *Lamarckiana* hybrid, as ROSENBERG[10a] has found in the apogamous species of Hieracium, the number of chromosomes is much higher than in other species of the genus examined. In the apogamous species in various genera investigated in recent years, where the chromosome numbers are known, the number of chromosomes in apogamous species is nearly or exactly double the sporophyte number in closely related, normally fertilized members of the same genus. A list of these cases need not be given. There may be a suggestion here as to how variation in the chromosome numbers of a genus originates. The same author[19] finds that in *H. excellens* the egg cells have different numbers of chromosomes, owing to irregularities in the reduction divisions. When this form is crossed with another species, the hybrids of the F_1 have different numbers of chromosomes. Whether this is the cause of the different types in the F_1 is not ascertained.

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[18] ROSENBERG, O., Ueber die Tetradentheilung eines Drosera-Bastardes. Ber. Deutsch. Bot. Gesells. **22**:47–53. *pl. 4.* 1904.

[19] ROSENBERG, O., Cytological investigations in plant hybrids. Rep. 3d Internat. Conf. on Genetics. Jour. Roy. Hort. Soc. London **24**:289–291. 1906.

It seems probable, or at least possible, that there is a connection between the type of hybridization in Oenothera and the chromosome numbers, though just what that connection may be is at present quite unknown. Apparently whatever determines the twenty or twenty-one chromosomes determines also that the parent shall have the *O. Lamarckiana* characters. There is thus a possibility that this may be found to have a bearing upon the cause of dominance and recessivity in Mendelian hybrids, the characters of the individual being determined in this case by the presence or absence of the extra chromosomes. CANNON'S[20] suggestion that the Mendelian segregation of characters results from the segregation of maternal and paternal chromosomes in the reduction mitoses is as yet a hypothesis without proof, though ROSENBERG'S[21] recent paper, in which he states that in the pollen development of the Drosera hybrid occasionally two pollen grains of the tetrad have the morphology of one parent and two that of the other, shows that in some cases, at least, segregation of characters takes place at this point in the life history.

Practically the same theory of the segregation of the characters and purity of the germ cells in plant hybrids on the basis of the chromosomes (idants) was put forward by WEISMANN[22] in 1892, in connection with his theory of the germ-plasm. At this time the Mendelian hybrids had not been rediscovered, and complete segregation of the characters of the parental types was regarded as a rare occurrence in hybrids. It may be worth while to quote WEISMANN'S statement (p. 299):

> The germ-mother cells of the hybrid contain a group of idants (chromosomes) derived from the paternal, and another from the maternal ancestral species. If therefore the "reducing-division" halves the germ-plasm of these mother-cells in

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[20] CANNON, W. A., A cytological basis for the Mendelian laws. Bull. Torr. Bot. Club **29**:657–661. *fig. 1.* 1902.

[21] ROSENBERG, O., Erblichkeitsgesetze und Chromosomen. Botaniska Studier, Upsala, pp. 237–243. *figs. 5.* 1906.

[22] WEISMANN, AUGUST, The germ-plasm. English translation. New York. 1898.

> such a manner that idants of the mother alone reach one ripe germ-cell, and those of the father alone are contained in another, it is possible that two such germ-cells may unite when fertilization takes place between these hybrids. In such a case a plant completely resembling one of the ancestral forms would arise, for it would have been produced from a germ-plasm which contains idants of this species only.

He concludes, however, (on a theoretical basis only) that this rarely happens.

To obtain the Mendelian ratios in hybrids on the basis of the purity of the germ-cells, it is necessary to assume that the mechanism of the reduction mitoses regularly segregates the maternal and paternal chromosomes, and that their distribution to the daughter cells in such cases is not merely a matter of chance, or that a part merely of the chromosomes from one parent can determine the dominance of that form. ROSENBERG[23] has elaborated such a scheme of chromosome segregation in connection with his study of *Drosera rotundifolia* × *D. longifolia*. He found that in the metaphase of the heterotypic mitosis in the pollen mother cell the chromosomes of the two parents, which can be distinguished by their size, are not all oriented, with for example the paternal chromosomes toward one pole of the spindle in the equatorial plate, but some from each parent will enter both daughter nuclei, their orientation on the spindle being thus determined by chance. The Drosera hybrid does not show complete dominance, but is intermediate, some of the characters being nearer to either parent. The character of the hybrid in later generations has not yet been determined.

DEVRIES'[24] conception of the segregation in Mendelian hybrids is that where the parents differ in one unit character this character will be represented in the hybrid by a single *Anlage* from each parent. If these *Anlagen* are assumed to be each represented by a single chromosome, the corresponding members of the other chromosome pairs being qualitatively alike, this would give, as ROSENBERG[23] points out, a chromosome basis for Mendelian segregation of characters. In the formation of the megaspores and microspores, half would contain the chromosome representing the peculiar paternal character, and half the chromosome representing the maternal. The germ-cells would thus all be pure in regard to this character, and in self-fertilization of the hybrid, according to the laws of chance the usual percentages of a Mendelian hybrid would arise.

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[23] ROSENBERG, O., Ueber Reduktionstheilung in Drosera. Meddel. Bot. Inst. Stockholm, pp. 13. *figs. 20.* 1904.

[24] DEVRIES, HUGO, Befruchtung und Bastardierung. Leipzig. pp. 62. 1903.

OTHER CYTOLOGICAL OBSERVATIONS

Several interesting cytological observations, a detailed description of which will be presented at a later time, may be briefly stated here. In the heterotypic mitosis in the pollen mother cells of the *Lamarckiana* hybrid, the usual split in the chromosomes, in the anaphases, which is generally described as a premature split for the homotypic mitosis and is believed by many cytologists to be the reappearance of a split which occurred at the time of synapsis, occurs as described in my former paper. In the early telophases, before there is any indication of a nuclear membrane around the daughter nuclei or of the beginning of a passage into the semi-resting condition, which occurs later, chromosomes having the appearance of distinct tetrads are frequently found. The indications are that this tetrad appearance, which is often very clear and characteristic, may be due to the two halves of a daughter chromosome (which were formed by the split in the anaphases of the heterotypic) remaining side by side and each becoming clubbed at the ends, the portion between these ends in each remaining only as a very delicate connecting thread. This gives the appearance of four spherical bodies of approximately equal size, connected in pairs in one direction by two parallel delicate threads. In other cases the tetrads have quite a different shape, having rather the appearance of a hollow ring drawn out sharply at the four corners. In still other cases, in this telophase, chromosomes are seen having the shape of a pair of spheres close together, or a dumb-bell, and often chromosomes are found at this stage showing no indication of even a double nature, but appearing univalent. This variation in form does not obscure the distinctness of the chromosomes, which are usually clearly distinguishable from each other at this time, and easily counted. All these different forms may be found together in the same nucleus, though so far as observed one form usually predominates in a given telophase. Figures showing these appearances will be published later.

In the homotypic mitosis, as observed in *O. Lamarckiana*, the chromosomes usually become two-lobed in the anaphases. Just how this shape arises from the pear-shaped chromosomes of the early anaphase has not been determined. In the telophase of this mitosis the chromosomes are nearly always two-lobed or dumb-bell-shaped and can be readily counted (seven in *O. Lamarckiana*) in this condition. They soon become more or less irregular in outline, however, as the nuclei pass into the resting condition, but this characteristic shape usually persists for a short time at least after the formation of the nuclear membrane.

Several interesting features have appeared in connection with the examination of nuclear divisions, in various somatic tissues, a work which is not yet completed. All the tissues examined are flower tissues about the time when the reduction divisions are taking place in the pollen mother cells or later. The conditions so far as observed are identical in corresponding tissues of the three forms examined (*O. lata*, *O. Lamarckiana*, and the *Lamarckiana* hybrid), except for the difference in the number of chromosomes. In the tissue of the anther wall and in the rapidly dividing tissues of the young petals, in both of which a great many chromosome counts were made, the chromosomes are crescents, rods, or loops in metaphase and the early anaphases, the apices of the loops converging in telophase to the poles of the spindle. In the telophase these chromosomes also become two-lobed or dumb-bell-shaped. This shape is very characteristic and apparently constant, or at least occurs very generally at this stage. Observations which are as yet incomplete indicate that in the early telophase these chromosomes begin to appear two-lobed. This shape is very clear a little later, about the time the membrane of the daughter nucleus is formed. The chromosomes can be distinctly counted at this time, and the number is apparently constant in each form. It seems probable that this clubbing of the ends of the chromosomes to form dumb-bells or two-lobed chromosomes in the telophase of the mitoses in these tissues is the same thing that happens in the telophase of the heterotypic mitosis, giving rise to the tetrad appearance, and in the telophase of the homotypic, giving rise to the two-lobed or dumb-bell-shaped chromosomes. Whether this lobing of the chromosomes giving a bivalent appearance is in all these cases a preparation for the split in the next mitosis cannot at present be determined. The paucity of investigations on the somatic mitoses in plants leaves open the possibility that some of the phenomena of the reduction mitosis to which a special significance has been attached may find their counterpart in ordinary somatic mitoses. It is believed that further study will settle the interpretation of these appearances.

Measurements have shown that the size of the cells, nuclei, and chromosomes in the anther wall is on the average approximately the same as in the petals, though there is considerable variation in the size of the cells of the latter. It is interesting to note that while the chromosomes in the somatic tissues are loops in the metaphase and early anaphases, in the pollen mother cells the chromosomes are usually spherical or pear-shaped in these stages of both the reduction mitoses. This difference is very striking. In the telophase of the homotypic, however, the characteristic shape is apparently the same as in the corresponding stage of somatic mitoses, that is, two-lobed. Measurements indicate that the somatic chromosomes are at this stage considerably smaller than the homotypic chromosomes, though there appears to be much variation in the size of the former.

SUMMARY

1. In my previous paper the sporophyte number of chromosomes in *O. lata* was found to be fourteen, and in the *Lamarckiana* hybrid obtained by crossing *O. lata* with *O. Lamarckiana* pollen, at least twenty. The number of chromosomes is now found to be twenty or twenty-one constantly in all the plants of the *Lamarckiana* hybrid examined, while the number in the pure *O. Lamarckiana*,[25] as well as in *O. lata*, is fourteen, the reduced number being seven. These results are obtained from a large number of counts in various stages of the reduction mitoses and also in the somatic divisions (floral tissues). Thus the pure *O. Lamarckiana* and the *Lamarckiana* hybrid, which are identical in external appearance, differ widely in the number of their chromosomes.

2. The origin of the bodies called heterochromosomes, which are present in the reduction divisions in all the forms studied, is a matter of

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[25] J. M. GEERTS, in a short paper just received (Ueber die Zahl der Chromosomen von *Oenothera Lamarckiana*. Ber. Deutsch. Bot. Gesells. **25**:191–5. *pl. 6.* 1907), also finds that the sporophyte number of chromosomes in the pure *O. Lamarckiana* is 14 and the gametophyte number 7.

doubt, and until their mode of origin is ascertained no significance can be attached to them.

3. In the telophase of the heterotypic mitosis in the pollen mother cells of the *Lamarckiana* hybrid, the chromosomes frequently have distinctly the form of tetrads. In the homotypic mitosis of *O. Lamarckiana*, as well as in the somatic mitoses (in various floral tissues) in all forms studied, the chromosomes are two-lobed in the telophase. The tetrad appearance is probably due to the same lobing of the bivalent chromosomes that takes place in the telophase of the homotypic and somatic mitoses.

This investigation has been carried on under the supervision of Professor John M. Coulter and Dr. Charles J. Chamberlain.

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